Columbia University
in the City of New York
[NEW YORK 27, N. Y.]
DEPARTMENT OF ZOÖLOGY

Mar. 17 1950

Dear Josh,

By this time you have probably long since reached the conclusions that follow, but anyway here are my thoughts on the role of chromosome elimination in the killing of K12 diploids. In your letter you stated that a population 85% heterogenic became 25% heterogenic at 10% survival, and suggested that the chromosome might be the unit of inactivation, implying the sequence: diploid → haploid → dead.

In its simplest form, assuming independence of all events, your hypothesis fixes the ratio diploid:haploid:dead without the necessity of considering the relation between dose and the inactivation unit. Where p is the prob. that a chromosome is eliminated:

1) $(1-p)^2 + 2p(1-p) + p^2 = 1$

(diploid = $(1-p)^2$; haploid = $2p(1-p)$; dead = p^2)

The presence of 15% haploids at the beginning of the experiment is annoying but easily overcome:

2) $S = 1 - .15p - .85p^2$

Where S = .1,

3) $0 = .9 - .15p - .85p^2$

and,

4) $p = \dfrac{.15 - \sqrt{.0225 + 4(.85)(.9)}}{-1.7} = .944$

thus,

5) $P_{haploid} = .15(1-p) + 1.7p(1-p) = .15 + 1.55p - 1.7p^2 \approx 0.1$

and,

6) $P_{diploid} = .85(1-p)^2 = .85 - 1.7p + .85p^2 = .003$

In other words you should have found practically all haploids among 10% survivors to agree with theory.

Assuming that killing occurs only according to hypothesis, the expected survival with a haploid:diploid ratio of 3 is computed from (5), (6) and (2):

7) $\dfrac{.15 + 1.55p - 1.7p^2}{.85 - 1.7p + .85p^2} = 3 \quad \therefore \; 4.25p^2 - 6.65p + 2.4 = 0$

$$p = \frac{6.65 - \sqrt{44.2 - 40.8}}{8.5} = .565 \qquad S = .64$$

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Since the discrepancy between your survival and induced segregation necessitates the assumption of some additional killing mechanism, what would be the survival of this effect alone? Where S_1 is the survival of double chromosome elimination and S_2 of the unknown process, the observed survival is $S_1 S_2$ and,

8) $$S_2 = \frac{S_1 S_2}{S_1} = \frac{.1}{.64} = .156$$

Thus the dose would have given you only 16% survival even if none of the killing is due to the successive inactivation of chromosomes. You might try working it out for four strands, which would favor your hypothesis more although I think there would still be a large discrepancy.

This is the most I can squeeze out of the meagre data and I would certainly like to know more. My data on conidia of Neurospora heterokaryons tell the same story, there is a highly reproducible small incidence of induced segregation, about 1/10 as much as in K12 and quite unimportant as a cause of killing. In computing the expected heterokaryon:homokaryon ratio in Neurospora the use of the above approach would be like doing C.B.deMille's income tax, due to the distribution of nuclear number. Thus in rejecting the hypothesis that the nucleus is the unit of inactivation, I have used the approximation, good when p is large, that the proportion of conidia having at least two viable nuclei is given by

9) $$P_{\geq 2} = 1 - (e^{-m} + me^{-m})$$

where $e^{-m} = 1 - S$. The expression represents twice the maximum possible frequency of heterokaryons for a given survival. As you know, I get up to 50% balanced lethal heterokaryons but this will not account for enough killing to be experimentally detectable. Thus the unit of inactivation remains unidentified. To shorten a long story there are (at present) two types of units of inactivation one being present in about the same number as nuclei, the other in a much larger number. Both are non-genetic, at least in the sense that no extrapolation of the heritable changes in the survivors can account for the proportion killed. This does not necessarily mean that genetic material is not involved since we can easily imagine the paradox of a mutational change which is not inherited. To state it crudely what I have in mind is the type of change which would prevent a gene from performing its heterocatalytic function but not prevent it from conferring its original unmutated configuration on its sister homologue when and if the latter is formed. If there exist such functions as are immediately essential for the reduplication of the genome, the above process would cause permanent arrest in a haploid uninucleate cell. However, in a multinucleate cell recovery could occur unless all of the nuclei were affected, and following recovery no genetic changes need necessarily be observed among the progeny of any of the component nuclei.

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The attempt to identify the unit of inactivation in Neurospora is one of my main projects, thus your stuff becomes intensely interesting since it offers the possibility of generalizing the conclusions. Specifically I would like the answers to the following questions:

1) What is the shape of the survival curves and is there a difference between haploid and diploid?
2) What is the nature of the induced segregants, all parental, or some recombinant types?
3) How do you detect balanced lethals if they occur?

I solemnly promise to reply promptly this time.

We are going to have another baby in Sept. and plan to leave for Woods Hole about May 15. Barbee sends her love and says she throws together the chestnut stuff by instinct but the process is something as follows: Boil ca. 3 lb. chestnuts 15 min., remove shells and peelings, put edible portion through a meat grinder with fine head. Add about ¼ lb. butter and enough cream to produce a creamy consistancy when mixed (like mashed potatoes). Place in a casserole and heat thoroughly in a medium oven. Serves 8.

Let us know in advance if you are coming East.

Sincerely,

Kim

K.C.Atwood

P.S. Fast one is right. I don't see how it slipped by the referees.